International Journal of Infectious Diseases 40 (2015) 45–53

Contents lists available at ScienceDirect

International Journal of Infectious Diseases

journal homepage: www.elsevier.com/locate/ijid

Host biomarkers are associated with progression to dengue haemorrhagic fever: a nested case-control study

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ARTICLE INFO

Article history:
Received 19 April 2015
Received in revised form 28 July 2015
Accepted 30 July 2015

Corresponding Editor: Eskild Petersen, Aarhus, Denmark

Keywords:
dengue
biomarkers
pathogenesis
sICAM-1
soluble endoglin
CXCL10

ABSTRACT

Objectives: Dengue represents the most important arboviral infection worldwide. Onset of circulatory collapse can be unpredictable. Biomarkers that can identify individuals at risk of plasma leakage may facilitate better triage and clinical management.
Design: Using a nested case-control design, we randomly selected subjects from a prospective cohort study of dengue in Colombia (n = 1582). Using serum collected within 96 hours of fever onset, we tested 19 biomarkers by ELISA in cases (developed dengue hemorrhagic fever or dengue shock syndrome (DHF/DSS); n = 46), and controls (uncomplicated dengue fever (DF); n = 65) and healthy controls (HC); n = 15.
Results: Ang-1 levels were lower and angptl3, sKDR, sEng, sICAM-1, CRP, CXCL10/IP-10, IL-18 binding protein, CHI3L1, C5a and Factor D levels were increased in dengue compared to HC. sICAM-1, sEng and CXCL10/IP-10 were further elevated in subjects who subsequently developed DHF/DSS (p = 0.008, p = 0.028 and p = 0.025, respectively). In a logistic regression model, age (odds ratio (OR) (95% CI): 0.95 (0.92-0.98), p = 0.001), hyperesthesia/hyperalgesia (OR; 3.8 (1.4-10.4), p = 0.008) and elevated sICAM-1 (>298ng/mL: OR; 6.3 (1.5-25.7), p = 0.011) at presentation were independently associated with progression to DHF/DSS.
Conclusions: These results suggest that inflammation and endothelial activation are important pathways in the pathogenesis of dengue and sICAM-1 levels may identify individuals at risk of plasma leakage.

1. Background

Dengue is an emerging infectious disease of global importance with 2.5 to almost 4 billion people residing in dengue endemic countries in which an estimated 50-100 million infections occur annually.[1,2] Historically, the highest risk areas for dengue fever were in Southeast Asia and the western Pacific (where approximately 75% of the global disease burden is located). However, the risk of dengue, and severe dengue, is increasing in the Americas following disease re-emergence after lapsed vector control programs.[3] Between 2000 and 2006, the majority of cases reported (68%) were registered in this region.[4] Cyclical outbreaks of dengue in the Caribbean, and Central and South America have resulted in considerable morbidity and mortality. In 2013, 2.35 million cases of dengue were reported in the Americas, of which 37,687 cases were classified as severe dengue, with Brazil and Colombia recording the highest incidence.

Dengue is an important consideration in the differential diagnosis of fever in endemic countries and in travelers returning from these regions.[5] Dengue infection presents with a wide spectrum of signs and symptoms, including rapid onset of fever that may be accompanied by myalgia, arthralgia, retro-orbital pain, facial flushing, erythema, nausea, vomiting, cutaneous

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http://dx.doi.org/10.1016/j.ijid.2015.07.027

hyperesthesia or hyperalgesia, and minor bleeding abnormalities as evidenced by a positive tourniquet test. A small percentage of individuals with dengue will develop life-threatening complications, which can include plasma leak and circulatory collapse. The clinical evolution of disease can be unpredictable making the initial clinical assessment a critical step in patient management.[6]

Dengue is a single-stranded RNA virus with four distinct serotypes (DEN-1 to -4). Risk of haemorrhage and plasma leak is generally higher in secondary infections, but other factors including age, sex, and other host and viral determinants also contribute to disease susceptibility. The prevailing hypothesis is that cross-reactive, non-neutralizing antibodies in secondary heterotypic infections facilitate viral entry into target cells, thereby increasing viral load, enhancing deleterious inflammatory responses and contributing to endothelial activation and dysfunction (reviewed in[7]). The endothelium is central to dengue pathogenesis as increased vascular permeability and fragility are defining features in severe disease. However, inflammation, coagulation and complement activation are also involved in disease pathogenesis, and alterations in soluble mediators from these pathways have been described in severe dengue infections.[8–18]

Clinical management of dengue depends on appropriate triage, referral and treatment of cases, especially in outbreaks. Although there are no specific anti-infective or immunomodulatory therapies to prevent plasma leak, appropriate fluid management and supportive care can reduce the mortality rate from over 20% to less than 1%.[3,19] With rates of severe disease on the rise, especially in the Americas,[20–22] new tools are urgently needed to improve patient triage and risk prediction of severe disease.[23] We investigated whether host biomarkers, chosen from pathways implicated in dengue pathogenesis, were altered in dengue relative to healthy controls, and whether the biomarkers assessed during the acute phase of disease were associated with the subsequent development of dengue haemorrhagic fever (DHF) or dengue shock syndrome (DSS).

2. Methods

2.1. Study Population

All four dengue virus serotypes circulate in Colombia, which reports the highest number of severe dengue cases and deaths in the region.[3,24] Bucaramanga is a metropolitan area in northeastern Colombia where the incidence of dengue ranges from 113-269 cases per 100,000 people.[25–27] Participants from Bucaramanga who presented within 96 hours of fever onset were eligible for recruitment if they were greater than five years of age. Subjects were excluded based on the presence of the following conditions: history of concomitant diseases such as diabetes, acquired immunodeficiency syndrome (AIDS), hematologic disorders, cancer, or cardiac disease, albuminemia (<3 g/dL), evidence of severe dengue at presentation, for example major bleeding, effusions, or shock. After participants provided informed consent, a physical examination was performed, and a blood sample was obtained to determine hematocrit, albumin levels, and platelet and leukocyte counts. A serum sample was collected and stored at -80C for future biomarker assessment.

Diagnosis of dengue virus infection was confirmed based on viral isolation or serology (seroconversion from a negative to a positive IgM test or a four-fold increase in dengue antibodies in a convalescent blood sample). All study participants were enrolled before the development of DHF/DSS. Participants were followed daily until day 7 of disease with daily microhematocrit measurements to facilitate the recognition of severe dengue. Platelet counts were repeated daily for subjects with previous platelet counts less than 120,000/mm^3 or if there were signs of spontaneous haemorrhage, effusion, oedema or change in hematocrit > 10%. Study participants were classified as DF or DHF/DSS following recovery and full chart review using the 1997 WHO classification system according to the study design.[28]

2.2. Study Design

This study was a case-control study nested within the prospective cohort study (n = 1582) of suspected dengue in Bucaramanga, Colombia. Blood samples were collected from study participants at clinical presentation. Cases were individuals who developed DHF/DSS and controls had dengue fever (DF). Study subjects with available serum samples were eligible for biomarker testing and were randomly chosen from the cohort database using computer generated simple randomization. Serum samples were collected from 15 healthy Bucaramangan adults to derive a population-based normal range.

2.3. Biomarker Assessment

Serum concentrations of biomarkers were measured in samples collected at presentation using ELISA DuoSets from R&D Systems (Minneapolis, MN). Biomarkers measured were C-reactive protein (CRP), CXCL10/IP-10, IL-18 binding protein (IL-18BP), IL-10, chitinase 3-like 1 (CHI3L1), C5a, complement factor D, angiopoietin-1 (Ang-1), angiopoietin-2 (Ang-2), soluble Tie-1 (sTie-1), soluble Tie-2 (sTie-2), angiopoietin-like protein 3 (Angptl3), angiopoietin-like protein 4 (Angptl4), vascular endothelial growth factor (VEGF), soluble VEGF R1 (sFlt-1), soluble VEGF R2 (sKDR), soluble endoglin (sEng), soluble intercellular adhesion molecule-1 (sICAM-1) and platelet factor 4. All ELISAs were validated prior to use, and sample dilutions were optimized for each biomarker using a dilution curve of serum obtained from febrile subjects with dengue fever in Bucaramanga. ELISAs were tested according to the manufacturer's instructions (R&D Systems) with minor modifications previously described.[29]

2.4. Statistical Analysis

GraphPad Prism v5, SPSS v20 and MedCalc® v12 were used for statistical analysis. Comparisons of continuous variables were performed using the Mann-Whitney U test. Comparisons of proportions were performed using Pearson chi-square test or Fisher's exact test, as appropriate. Exploratory logisitic regression models were built using forward step-wise selection including all variables with $p < 0.10$ by bivariate analysis. Model fit was assessed using the Hosmer-Lemeshow test and ensuring the term was not significant $p > 0.05$. Biomarkers were dichotomized by generating a ROC curve and using the Youden index to identify the optimal cut-off (Youden index: J = max [sensitivity+specificity]-1).

3. Results

3.1. Clinical Characteristics of Subjects that Developed DHF/DSS

111 subjects with microbiologically confirmed dengue virus infection were included in the current study along with 15 healthy controls from Bucaramanga. 65 had DF while 46 developed DHF/DSS over the course of clinical follow up.[3] Individuals that developed DHF/DSS were significantly younger, had higher axillary temperatures and lower mean arterial pressure at presentation and were more likely to report chills, hyperesthesia/hyperalgesia, and dizziness than those with DF (Table 1). The median day of illness at presentation was day 4 for both groups (p = 0.461,

Table 1
Demographic and clinical features of cohort at presentation

	DF (n = 65)	DHF/DSS (n = 46)	P value
Demographics			
Age, years	28.0 (19.0-45.5)	21.0 (14.0-31.3)	0.006
Sex (% F)	35 (53.8)	25 (54.3)	0.958
Height, cm	163 (155-172)	160 (150-168)	0.094
Weight, kg	63.0 (54.0-76.5)	59.0 (47.0-70.0)	0.156
Duration of fever, hours	77.0 (66.8-89.0)	75.3 (60.1-88.8)	0.733
Laboratory			
Axillary temperature, °C	36.3 (36.0-37.0)	36.8 (35.9-37.9)	0.067
Platelet count (x10^3 μl/mL)	126.0 (83.0-182.0)	119.5 (79.8-176.3)	0.940
Leukocyte count	2900 (2100-4000)	2950 (2275-3925)	0.682
Hematocrit	39.4 (36.1-42.6)	38.0 (35.5-42.0)	0.436
Mean arterial pressure	88.0 (80.7-94.0)	83.7 (76.7-90.8)	0.038
Tourniquet test (# petechiae)	22.0 (4.5-32.5)	17.5 (5.0-25.0)	0.281
Aspartate aminotransferase [a]	59.9 (40.0-114.8)	56.5 (43.0-95.0)	0.958
Alanine aminotransferase [a]	37.6 (22.0-57.0)	30.7 (24.0-74.0)	0.889
Lactate dehydrogenase [b]	320.0 (200.5-433.1)	333.8 (172.0-716.9)	0.540
Albumin [c]	4.5 (4.2-4.7)	4.1 (3.8-4.5)	0.069
Creatinine phosphokinase [c]	134.6 (59.8-769.0)	76.6 (54.1-184.5)	0.170
Cholesterol [d]	155.1 (141.0-164.0)	148.6 (113.3-154.6)	0.118
Trigylcerides [d]	121.7 (87.2-175.2)	159.1 (72.7-203.3)	1.000
Signs and Symptoms (%)			
Headache	61 (93.8)	44 (95.7)	0.678
Retro-orbital pain	43 (66.2)	35 (76.1)	0.259
Asthenia	46 (70.8)	31 (67.4)	0.704
Muscle Pain	59 (90.8)	41 (91.1), n = 45	0.951
Joint Pain	52 (80.0)	36 (80.0), n = 45	1.000
Chills	64 (98.5)	41 (89.1)	0.032
Sore throat	23 (35.4)	12 (28.3)	0.430
Facial erythema	35 (53.8)	22 (47.8)	0.532
Rash	25 (38.5)	23 (53.5), n = 43	0.124
Hyperesthesia/hyperalgesia	15 (23.1)	17 (40.5)	0.055
Nausea	47 (72.3)	38 (82.6)	0.207
Vomiting	20 (30.8)	18 (39.1)	0.360
Diarrhea	25 (38.5)	16 (34.8)	0.692
Abdominal pain	31 (48.4)	30 (65.2)	0.081
Blurred vision	24 (36.9)	20 (44.4)	0.429
Dizziness	39 (60.0)	35 (77.8)	0.051
Conjunctival injection	31 (47.7)	17 (37.0)	0.261
Orthostatic hypotension	8 (12.3)	9 (19.6)	0.296

Participants with dengue fever (DF) that did not develop complications, DF participants that developed DHF/DSS.
[a] DF (n = 40), DHF/DSS (n = 31); [b] DF (n = 38), DHF/DSS (n = 31); [c] DF (n = 24), DHF/DSS (n = 18), [d] DF (n = 15), SD (n = 13).

chi-square) and the median time to DHF/DSS diagnosis was 2 days after presentation.

3.2. Alterations of Host Biomarkers in Dengue

We evaluated 19 host biomarkers in patients with dengue virus infection and compared them to healthy controls to determine which markers were elevated during the acute febrile phase of dengue (2-4 days after symptom onset). Biomarkers were broadly chosen from four pathways implicated in dengue pathogenesis: inflammation, endothelial activation, complement activation, and coagulation. Eleven biomarkers differed in dengue infection compared to healthy controls ($p < 0.05$ by Mann-Whitney U test, Table 2). Ang-1 was significantly reduced while Angptl3, sKDR, sEng, sICAM-1, CRP, CXCL10 (IP-10), IL-18 binding protein, CHI3L1, C5a and Factor D were increased in dengue fever compared to healthy controls (Figure 1).

3.3. Host Biomarkers are Associated with the Development of Hemorrhage

We hypothesized that host biomarkers altered in dengue precede clinical manifestations of hemorrhage and vascular leak. Three biomarkers were significantly elevated at presentation in subjects that subsequently developed DHF/DSS compared to those with an uncomplicated disease course: sICAM-1, sEng and CXCL10 ($p = 0.008$, $p = 0.028$ and $p = 0.025$ respectively) (Table 2, Figure 2). Levels of CXCL10 were significantly correlated with sICAM-1 levels (Spearman's rho, p-value; 0.468, $p < 0.001$) and sEng levels (0.348, $p < 0.0001$), but there was no statistically significant association between sEng and sICAM-1 (0.184, $p = 0.053$).

We explored the relationship between the biomarkers associated with disease progression and routine clinical laboratory values at the time of clinical presentation, including platelet and leukocyte counts, hematocrit, bleeding tendency, mean arterial pressure and liver enzymes. Levels of sICAM-1 and sEng correlated weakly and negatively with the platelet count (Spearman's rho, p-value: sICAM-1, -0.197, $p = 0.038$; sEng, -0.219, $p = 0.021$). CXCL10 correlated with both platelet and leukocyte counts (platelet count, -0.533, $p < 0.0001$; leukocyte count, -0.355, $p < 0.0001$). Biomarker levels were not correlated with hematocrit, the number of petechiae by tourniquet test or mean arterial pressure. Both sICAM-1 and CXCL10 were significantly correlated with AST and ALT (AST: sICAM-1, 0.375, $p = 0.001$; CXCL10, 0.314, $p = 0.008$; ALT: sICAM-1, 0.410, $p < 0.0001$; CXCL10, 0.287, $p = 0.015$). sEng did not correlate with AST or ALT but was negatively correlated with total cholesterol and low density lipoproteins (cholesterol, -0.465, $p = 0.013$; low density lipoprotein, -0.601, $p = 0.001$).

3.4. Predictive Models of DHF: Integrating Clinical and Biomarker Data

Based on the hypothesis that host biomarkers can add value to clinical findings, we explored models to integrate our biomarker

Table 2
Biomarkers in Dengue

	HC (n = 15)	Dengue	
		DF (n = 65)	DHF/DSS (n = 46)
Inflammation			
CRP [a]	1.8 (2.3-6.1)***	18.7 (9.2-41.3)	21.0 (6.4-41.4)
IP-10	0.2 (0.2-0.4) ***	2.8 (1.0-4.4)	3.6 (1.8-5.0) †
IL-18 BP	5.8 (4.3-8.3) ***	62.3 (36.4-97.0)	75.9 (52.8-90.6)
IL-10 [b]	0.03 (0.02-0.2)	145.2 (19.5-390.9)	58.0 (19.5-357.9)
CHI3L1	44.5 (35.8-61.3)*	61.6 (44.6-81.2)	57.3 (40.3-78.3)
Complement System			
C5a	18.5 (13.8-22.1) ***	53.8 (33.9-62.8)	52.5 (35.3-67.4)
Factor D [a]	1.1 (1.0-1.3)**	1.4 (1.1-1.6)	1.3 (1.2-1.6)
Endothelium			
Ang-1	44.9 (33.0-53.6)*	32.2 (25.6-43.9)	31.6 (22.3-47.0)
Ang-2	1.4 (1.0-2.6)	1.7 (1.3-2.4)	2.1 (1.3-2.9)
sTie-1	8.3 (6.8-12.7)	10.5 (8.1-12.9)	9.6 (8.1-13.5)
sTie-2	8.7 (6.4-9.7)	7.3 (5.5-8.9)	8.1 (6.0-9.9)
Angptl3	108.4 (82.3-117.5) ***	164.0 (140.6-192.9)	170.8 (146.5-196.3)
Angptl4	44.7 (30.2-90.9)	37.8 (29.3-56.3)	41.7 (33.6-64.2)
VEGF [b]	0.2 (0.2-0.3)	128.2 (48.8-291.9)	124.3 (48.8-321.5)
sFlt-1 [b]	39.1 (39.1-343.5)	39.1 (39.1-863.3)	94.6 (39.1-1074)
sKDR	5.3 (4.5-6.0) *	6.1 (5.5-7.1)	6.1 (5.5-7.1)
sEng	6.6 (5.7-8.4) ***	11.0 (9.2-13.2)	12.8 (10.3-14.7) †
sICAM-1	169.3 (135.8-287.3) ***	329.1 (284.1-467.1)	399.9 (323.0-539.0) ††
Coagulation			
Platelet factor 4 [a]	18.5 (15.6-27.1)	25.7 (16.2-41.2)	23.8 (15.6-37.7)

Healthy controls (HC), Participants with dengue that did not develop complications (DF), participants with dengue that developed DHF/DSS (DHF/DSS).
Biomarker concentration in ng/mL unless otherwise indicated, [a] μg/mL, [b] pg/mL.
*** $p < 0.001$ (HC vs. all dengue), ** $p < 0.01$ (HC vs. all dengue), * $p < 0.05$ (HC vs. all dengue).
†† $p < 0.01$ (DF vs. DHF/DSS), † $p < 0.05$ (DF vs. DHF/DSS).

findings with clinical data. All variables with $p < 0.10$ from bivariate analysis (Table 1) were considered for the predictive models and biomarkers were included as dichotomous variables with the following cut-offs (CXCL10 >1.07ng/m; sEng >11.8 ng/mL; sICAM-1 >298.2 ng/mL).

Using forward step-wise logistic regression, we developed a clinical model consisting of age and hyperesthesia/hyperalgesia (Table 3). We then added the biomarkers into the clinical model to generate a biomarker model that included age, hyperesthesia/hyperalgesia and sICAM-1. In the biomarker model, age was associated with a 0.95 (95% CI, 0.92-0.98, p = 0.002) odds of DHF/DSS while the presence of hyperesthesia/hyperalgesia was associated with 3.8-fold increased odds of developing DHF/DSS (95% CI, 1.4-10.4, p = 0.008). sICAM-1 >298.2 ng/mL was associated with a 6.3-fold increased odds of developing DHF/DSS (95% CI, 1.5-25.7, p = 0.011) (Table 3).

4. Discussion

Dengue fever is an emerging infectious disease endemic to the tropics and sub-tropics that causes cyclical outbreaks or epidemics that can overwhelm health care resources. Only a small proportion of infections will progress to severe disease (DHF/DSS), but it is difficult to accurately identify individuals at risk of plasma leak and circulatory collapse during the acute phase of disease when individuals are likely to first seek medical care. In this study, we investigated whether host proteins derived from pathways implicated in the pathogenesis of dengue would differentiate between subjects with an uncomplicated disease course versus those that developed DHF/DSS. Compared to healthy controls, eleven proteins were altered in dengue infection (ang-1, angptl3, sKDR, sEng, sICAM-1, CRP, CXCL10/IP-10, IL-18BP, CHI3L1, C5a, Factor D), and three proteins were significantly elevated at presentation in individuals who subsequently developed DHF/DSS (sEng, sICAM-1 and CXCL10). sICAM-1 was an independent predictor of DHF/DSS (Figure 2, Table 3). These results suggest that host biomarkers, once validated, may have clinical utility in patient triage and risk-stratification for referral, admission and implementation of timely supportive measures.

Consistent with other studies, levels of sICAM-1, CRP, CXCL10, C5a and Factor D were elevated and levels of Ang-1 were reduced in subjects with dengue.[11,12,16,30–36] IL-10 was neither elevated in dengue nor was it associated with the development of DHF/DSS in this population unlike findings reported from Venezuela;[37,38] but consistent with a recent study from Sri Lanka.[39] There were no differences in levels of VEGF or its receptors in predicting DHF/DSS, unlike a report from Thailand.[40] These discrepancies may reflect age differences;[41] however, other factors (host and viral genotype or the kinetics of infection) may also be involved.

This study identified four novel biomarkers associated with dengue infection: CHI3L1, IL-18 BP, Angptl3, and sEng. CHI3L1 is expressed by a variety of cells and tissues (including leukocytes and endothelium) in response to inflammatory stimuli and is involved in modulating inflammatory responses (reviewed in[42]). Elevated levels of CHI3L1 have been associated with dysregulated inflammatory responses in other infections such as pneumonia and malaria.[43–45] IL-18BP binds and neutralizes IL-18, a pro-inflammatory cytokine that is induced by and further induces expression of IFN-γ. IL-18 has been implicated in resistance to dengue infection in a murine model,[46] but IL-18 has also been associated with dengue disease severity in humans.[17] Angptl3 is a protein expressed by the liver and is involved in regulating angiogenesis and lipid metabolism;[47–49] however, Angptl3 levels were not associated with levels of cholesterol (total, HDL or LDL) triglycerides or liver enzymes AST or ALT in this study.

Endoglin (Eng) is an integral membrane protein that serves as a co-receptor for protein members of the transforming growth factor (TGF)-ß family. It is predominantly expressed on endothelium and is highly expressed at sites of active angiogenesis (including inflammation and vascular injury).[50,51] sEng is generated through proteolytic processing of membrane bound Eng through matrix metalloproteinase-14[52] and regulates vascular tone and permeability in addition to modulating immune responses.[53] Overexpression of sFlt-1 and sEng leads to increased microvascular

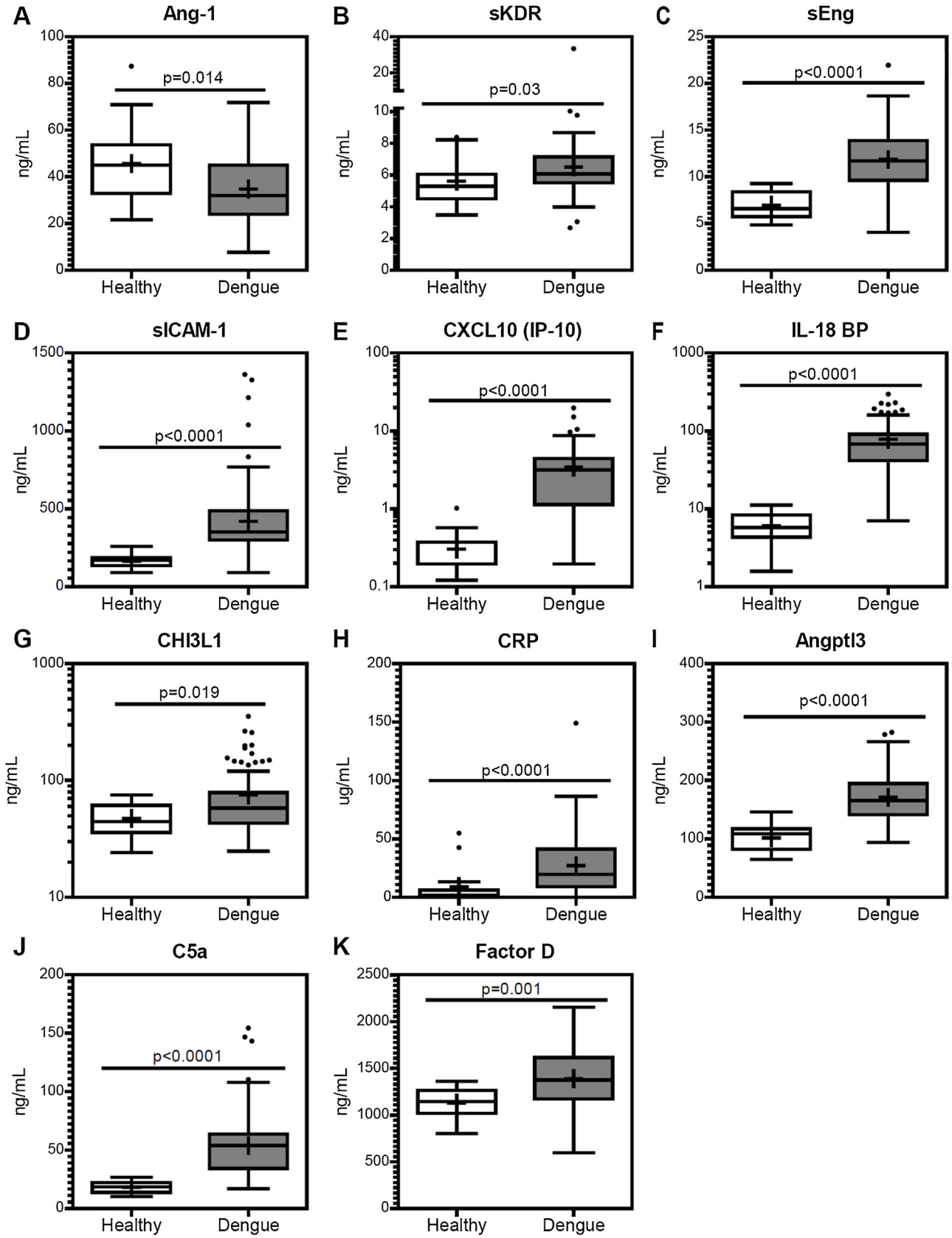

Figure 1. Host biomarkers that are altered in dengue infection.
(A-K) Box and whisker plots showing the distribution of biomarkers significantly different ($p < 0.05$) in healthy controls from Bucaramanga (n = 15) compared to subjects with serological confirmation of dengue fever (n = 111). Boxes show the median and interquartile range while the whiskers denote the minimum/maximum greatest value excluding outliers (defined as values 1.5x greater than the 3rd quartile or 1.5x less than the 1st quartile). The mean is denoted by the plus-sign (+). Data were analysed using the Mann-Whitney U test.

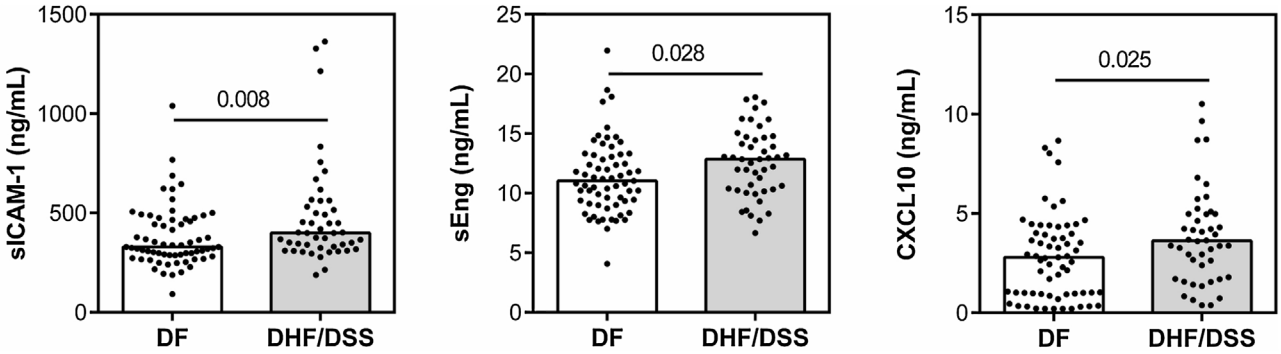

Figure 2. Biomarkers associated with the development of DHF/DSS.
Median and scatter of serum biomarkers (sICAM-1, sEng, CXCL10) measured at presentation in dengue infection in participants with uncomplicated dengue fever (DF, n = 65) and those who developed DHF/DSS (n = 46). Data were analysed using Mann-Whitney U test.

permeability in vivo and has an additive effect on increasing microvascular permeability in an organ specific manner.[53] Elevated sEng levels have been reported in a number of infectious and non-infectious conditions associated with vascular dysfunction including malaria and preeclampsia.[53–57] In this study, sEng levels were higher in individuals that subsequently developed DHF/DSS compared to those with DF. These findings are consistent with reports of sEng as a vascular permeability factor, but conflict with a recent study that found no difference in circulating sEng levels in dengue patients with plasma leakage assessed by X-ray or ultrasound on admission.[58] It is also possible that sEng may be modulating inflammatory responses through its effects on TGF-β signalling.

CXCL10 is an IFN-γ induced proinflammatory chemokine that is highly expressed by primary cells infected with dengue virus[34,59,60] and by primary cells from dengue-infected patients.[61] CXCL10 promotes viral clearance by binding to heparin sulfate, a co-receptor for viral entry,[62,63] and peak levels of CXCL10 have been temporally associated with maximal virus production from mononuclear cells.[64] In murine models, CXCL10 is associated with resistance to dengue infection.[63,65] CXCL10 levels are elevated during the acute febrile phase of infection[34,35,66] and are higher in dengue than other febrile illnesses.[33,34] There are little human data on CXCL10 levels and disease severity. A report from Colombia noted increased expression of CXCL10 in the cardiac tissue of a fatal case of dengue haemorrhagic fever,[67] but this finding requires validation. In this study, CXCL10 levels were higher in individuals that developed DHF/DSS. It is possible that CXCL10 was upregulated as an anti-viral response and individuals that developed DHF/DSS had higher viral loads, consistent with other reports.[68–70] Further studies are required to determine whether CXCL10 in humans is associated with resistance by reducing viral load or whether it may exacerbate disease by amplifying proinflammatory responses.

ICAM-1, a member of the immunoglobulin superfamily, exists as both a transmembrane (ICAM-1) and a soluble protein (sICAM-1). ICAM-1 is expressed on a variety of cells including leukocytes, hepatocytes and endothelial cells and is upregulated by inflammatory stimuli. Increased expression of ICAM-1 has been reported in dengue fever on endothelium[18,71–74] and leukocytes.[75,76] Upregulation of endothelial ICAM-1 occurs upon stimulation with sera from dengue patients, supernatants from dengue-infected cells or via direct stimulation with virus antigen in vitro. Stimulation occurs, at least in part, in a TNF and NF-κB-dependent manner.[18,71–73] Elevated sICAM-1 levels have been reported in dengue fever but have not been previously associated with disease severity,[30,31,75,77] except in a study by Bethell et al., where sICAM-1 was decreased in Vietnamese children with dengue shock syndrome. In the latter study, the decrease was attributed to a loss of protein secondary to capillary leak that occurs in severe dengue.[78]

In this study, sICAM-1 levels were increased in individuals with dengue infection compared to healthy controls (Figure 1), and sICAM-1 levels were higher in individuals that subsequently developed DHF/DSS compared to individuals with an uncomplicated course of DF (Figure 2). This effect was most pronounced when samples were collected prior to defervescence. sICAM-1 levels were positively correlated with liver enzymes AST and ALT and negatively correlated with platelet counts. Decreased platelet counts and increases in AST have been associated with severe disease onset in dengue fever,[79,80] but were not predictive in this nested case-control study, which may reflect a lack of power. sICAM-1 was an independent predictor of DHF/DSS in a clinical model alongside age and hyperesthesia/hyperalgesia and improved clinical prediction. These findings suggest that early systemic inflammation in dengue may lead to upregulation of cell-bound ICAM-1 and trigger cleavage of sICAM-1. The kinetics and functional consequences of elevated sICAM-1 in dengue infection require further investigation.

Table 3
Clinical and Biomarker Logistic Regression Models to Predict DHF/DSS

	B (S.E.)	Wald	Df	P Value	OR (95% CI)
CLINICAL					
Age	-0.047 (0.015)	9.397	1	.002	0.95 (0.93-0.98)
Hyperesthesia/hyperalgesia	1.184 (0.480)	6.088	1	.014	3.3 (1.3-8.4)
Constant	0.471 (0.432)	1.185	1	.276	1.6
BIOMARKER					
Age	-.052 (0.016)	10.733	1	.001	0.95 (0.92-0.98)
Hyperesthesia/hyperalgesia	1.345 (0.511)	6.933	1	.008	3.8 (1.4-10.4)
sICAM-1 (>298.2ng/mL)	1.837 (0.719)	6.523	1	.011	6.3 (1.5-25.7)
Constant	-0.998 (0.748)	1.782	1	.182	0.37

Coefficient, B; standard error, (S.E.); degrees of freedom, Df; odds ratio, OR: confidence interval, CI.
CLINICAL: Hosmer-Lemeshow (Chi-square, 12.506; df, 7; p = 0.085), -2 Log Likelihood (128.383), Cox & Snell R^2 (0.131).
BIOMARKER: Hosmer-Lemeshow (Chi-square, 11.008; df, 8; p = 0.201), -2 Log Likelihood (120.058), Cox & Snell R^2 (0.196).

Dengue has significant health, economic and social impacts on affected communities.[81] On average, a hospitalized case of dengue fever costs three times that of an ambulatory case,[82] and tools that can facilitate better patient triage at time of presentation with fever may reduce unnecessary hospital admissions and support the rational allocation of resources. A number of clinical signs and symptoms, laboratory parameters and biochemical markers have been associated with increased risk of clinical deterioration in dengue infection. However, the predictive accuracy of individual parameters varies according to the population studied and epidemiologic risk factors, and viral genotype. Host genetics may also affect host susceptibility to severe disease. The majority of published papers focus on paediatric populations from Southeast Asia; less is known about disease progression and markers of disease severity in adult populations and populations from Latin America. As Colombia has one of the highest rates of severe dengue and associated mortality in the western hemisphere[24,83], it is important to identify markers that can facilitate improved patient triage in this setting. In our study we found that patient age and the presence of cutaneous hyperesthesia/hyperalgesia, an under-recognized clinical symptom, were independently associated with progression to DHF/DSS.

Of interest, two candidate biomarkers associated with DHF/DSS progression in this study (CXCL10 and sICAM-1), are associated with disease severity in other viral haemorrhagic fevers. Increases in serum sICAM-1 and CXCL10 are associated with Crimean-Congo haemorrhagic fever (CCHF), and haemorrhagic fever with renal syndrome,[84–87] and sICAM-1 has been identified as a predictive marker of CCHF mortality.[84] Serum CXCL10 has been found to be significantly higher in patients with fatal yellow fever and fatal Zaire virus-associated Ebola virus disease (EVD); however, the prognostic value was not assessed in these studies.[88,89] Increases in sICAM-1 have been associated with fatal outcomes in Ugandan children with Sudan virus-associated EVD infection.[90] In the same outbreak, both sICAM-1 and CXCL10 were elevated in patients with haemorrhage.[91] These data suggest that the biomarker candidates identified in our cohort may also identify patients at risk of complications in other viral infections associated with endothelial pathobiology and haemorrhage.

This study represents one of the larger studies to assess the association of biomarkers with disease progression in samples prospectively collected during acute disease. Although the results are encouraging, they require validation in a larger prospective study and longitudinal sampling over the course of illness to evaluate biomarker kinetics. These findings also need to be validated using the new WHO dengue classification system to determine how the biomarkers relate to warning signs, organ dysfunction, and the development of severe dengue. Although further mechanistic studies are required to elucidate the role, if any, of the markers on mediating disease, our findings suggest there may be specific immune signatures that precede clinical deterioration. Further investigations into these pathways may identify novel markers to assist in patient triage, and inform the design of new interventions.

Acknowledgments

The authors wish to express their appreciation to all of the study participants and study staff.

Competing Interests: The authors declare that they have no competing interests.

Authors' Contributions: ALC, MG, MH, NR, VT, WCL, LAVC and KCK participated in the design of the study. ALC, MG and NR carried out the immunoassays. ALC and MH performed the statistical analysis. WCL, LAVC and KCK contributed reagents/ materials/ analysis tools. ALC, MH, VT and KCK wrote the initial draft of the manuscript. MG, NR, VT, WCL and LAVC critically revised the manuscript. All authors read and approved the final manuscript.

Funding: This work was supported by Colciencias [1102-459-21561 to LAVC] and the Canadian Institutes of Health Research [MOP-115160, MOP-136813 and MOP-13721 to KCK; Canada Research Chairs to KCK and WCL; Clinician Scientist Training Award to MH; Fellowship award to ALC]. The funders had no role is study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Ethics Statement: Ethical approval for this study was granted from the Medical Ethics Committee of the Universidad Industrial de Santander in Bucaramanga, Colombia. All subjects (or parents/ guardians for minors) provided written informed consent to enter the study. Selection of subjects for biomarker testing and subsequent biomarker data analysis was anonymous.

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